REMARKS

Applicant thanks the Examiner for acknowledging that claim 8 is a linking claim, and that the allowance of withdrawn claims 49, 53, 56, 58, 61, 63, 64, 66 and 68 will be considered at the time of allowance of claim 8. Therefore, claims 53, 56, 58, 61, 63, 64, 66 and 68 are withdrawn, but also currently amended, to conform to language in the independent claims 48, 55 and 60 from which they depend ("causing" is amended to "exposing" in all claims so listed).

Applicant also thanks the Examiner for withdrawing the enablement rejection for claim 10. Although claim 10 is cancelled herein, the limitation of claim 10 has been added to amended claims 8, 48, 55, 60 and new claims 70, 71, and 73.

Amendments to the Claims

Claims 8, 48, 55, and 60 are amended to add two new steps before "dissociating the embryoid bodies" of: providing chemically dissociated human embryonic stem (hES) cells (using a hES cell line from inner cell mass-stage human embryos - see Application p. 8, line 15 and p. 14, line 17); and aggregating the hES cells in suspension in the absence of LIF and bFGF to form embryoid bodies, wherein the cells of the embryoid bodies undergo initial differentiation (*id.*, p. 13, lines 32 – 34). Another new step is added after "dissociating the embryoid bodies" of: culturing said dissociated embryonic cells as a monolayer (original claim 10 and Application, p. 13, line 29 through p. 14, line 1).

In addition, claims 8, 48, 55 and 60 are herein amended to require the specific cell type (or human ectoderm, endoderm or mesoderm cells, respectively) to comprise a marker for terminally differentiated cells of the specific type, (or a marker for terminally differentiated human ectoderm, endoderm, or mesoderm cells, respectively).

Claim 10 is cancelled, but the limitation of claim 10 (culturing as a monolayer) is added herein to amended claims 8, 48, 55, 60 and new claims 70, 71 and 73.

New independent claims 70, 71 and 73 are added, analogous to claim 8, but the specific cell type is now identified as either human neuronal cells (claim 70), human

muscle cells (claim 71) or human pancreatic cells (claim 73). Dependent claim 72 specifically identifies the muscle cells as human cardiomyocytes.

Support for claim 70 is found in the application on pp. 17-19, Example 3, and Figures 6-8, where RT-PCR was used to assay for the presence of neurofilament light chain, dopamine receptor, and two serotonin receptors, and immunostaining confirmed the presence of neurofilament heavy chain protein. Additional support is found in the article by Carpenter, M., et al. entitled "Enrichment of Neurons and Neural Precursors from Human Embryonic Stem Cells" (*Exp Neurol* (2001) **172**:383-397), attached hereto as Exhibit B. The Carpenter et al. article verifies that using the methods disclosed in the present application, mature functional neurons can be obtain from undifferentiated hES cells.

As explained by Dr. Benvenisty (see Declaration 2, para. 10), "Carpenter et al. used undifferentiated hES cells (H1, H7 and H9 cells) to produce human EBs using a protocol nearly identical to mine, and then directed differentiation of dissociated ES from the EBs using retinoic acid [like Dr. Benvenisty] followed by culturing in differentiation medium (containing human neurotrophin-3 (hNT-3) and human brain-derived neurotrophic factor (hBDNF)). Analysis indicated that the resulting differentiated hES cell-derived neurons could synthesize neurotransmitters, respond to neurotransmitters, make synapses, and generate electrical activity (see pp. 390-392 and Figs. 3-7)."

Support for claims 71-72 is found in the application on p. 3, line 34 through p. 4, line 1; p. 9, lines 27-30; Figs. 3-4; and original claim 37. Moreover, using the methodologies detailed in the present application, Kehat et al. showed that hES cells can differentiate into functioning myocytes (*J Clin Invest* (2001)**108**(3):407-414 "Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes" – attached hereto as Exhibit C). As explained by Dr. Benvenisty (see Declaration 2, para. 11), "Kehat et al in the *J Clin Invest* article cite and follow my protocols for both formation of human EBs from hES cells (reference 12 - Itskovitz-Eldor et al. - in Kehat et al.) and directed differentiation (reference 13 - Schulbinder et al – in Kehat et al.) (see col. 1, p. 408 of Kehat et al.). Kehat et al. show in this paper that hES cell-derived differentiated myocytes form spontaneously contracting areas (see pp. 410 – 412, Figs. 1b, and 3-6)"

Moreover, as pointed out by Dr. Benvenisty "Following this initial study, Kehat et al. showed that hES cell-derived cardiomyocytes were able to form structural and electromechanical connections with cultured rat cardiomyocytes" (see Kehat et al., Nature Biotech (2004) 22(10):1282-1289 "Electromechanical integration of cardiomyocytes derived from human embryonic stem cells" – attached hereto as Exhibit D). See Declaration 2, para. 12. Again, the ground-breaking work of Dr. Benvenisty was followed and cited (see col. 1, p. 1282, citation 17 to Itskovitz-Eldor et al.), although in this paper, Kehat et al. used Dr. Benvenisty's protocols for generating embryoid bodies from hES cells and then differentiating the hES cells, but cited their previous 2001 *J. Clin. Invest.* paper for how to produce embryoid bodies and then differentiate the dissociated hES, rather than cite the primary reference - Dr. Benvenisty's PNAS paper. Nonetheless, as pointed out by Dr. Benvenisty "the two Kehat papers rely on my previous published procedures for generating EBs from hES cells and directing differentiation of dissociated hES cells, and verify that using my differentiation procedures, functional myocytes, particularly cardiomyocytes, are formed." *Id.*

Finally, support for claim 72 is found in the application on p.3, lines 33-34; p. 9, lines 31-34; Figs. 3-4; and original claim 37. And as with new claims 70-72 discussed above, other researchers have used the protocols of Dr. Benvenisty to direct differentiation of hES cells (by forming EBs and then dissociating and differentiating) that are functional pancreatic cells expressing insulin. See, for example, Assady et al. (*Diabetes* (2001) 50:1691-1697 "Insulin Production by Human Embryonic Stem Cells" – attached hereto as Exhibit E) wherein Dr. Benvenisty's protocols are cited as references 16 and 19 and his protocols are used to generate EBs (see "Research Design and Methods," p. 1692 of Assady, citing Dr. Benvenisty – ref. 19 – for production of EBs). As explained by Dr. Benvenisty, "Assady et al. specifically cite my PNAS paper, one of the publications that forms the basis of this application, as having used RT-PCR to detect a wide variety of differentiated cell markers, including insulin (see Assady et al., p. 1691, col. 1). Assady et al. then use protocols I developed for generating EBs from hES cells (*id.*, p. 1692, col. 2) and verify that one can direct differentiation of hES cells after forming EBs into human pancreatic cells that are functional, as verified by their ability to secrete insulin, and are probably also glucose responsive (although that could not be

absolutely confirmed because of the experimental parameters.)" See Declaration, para. 13.

In light of these supplemental references which confirm that the protocols disclosed in the present application, if followed, will in fact lead to functional differentiated cells, Applicant respectfully submits that there is support in the application for the amendments and new claims set forth with this response, and that the pending claims are fully enabled and meet the requirements of 35. U.S.C. § 112, para. 1.

New claims 74 and 75 are directed to a method for making human embryoid bodies and a method for making a culture of human embryonic cells from hEBs, respectively. Support for these new claims is found in original claim 8, and as detailed above for amended claim 8, in addition to support in the specification on p. 12, lines 16-17 and pp. 14-15, Example 1.

With these amendments and new claims, Applicant hereby submits that all pending claims have support in the specification and that no add new matter has been added.

Claim Rejections under 35. U.S.C. § 103(a) – Obviousness

The Examiner has upheld the obviousness rejections of claims 8, 9, 11, 12, 60 and 65 as being unpatentable over Thomson et al. (1998) *Science* **282**, 1145-1147 in view of Shamblott et al. (1998) *PNAS* (USA) **95**, 13726-13731 and further in view of Yuen et al. (1998) *Blood* **91**, 3202-3209. As the foundation for this rejection, the Office Action states on p. 4 that "Thomson teaches human ES cells that retain the ability to form all three embryonic germ layers," that Shamblott "teaches the production of human EBs from human primordial germ cells" and that Yuen teaches "the production of embryoid bodies in suspension from mouse ES cells."

As discussed in the interview of March 3, 2005, Shamblott et al. teaches production of hEBs from human primordial *germ* cells and Yuen et al. teaches the production of EBs in suspension from *mouse* ES cells, not human ES cells. As clearly explained by Dr. Benvenisty, "[h]uman EG cells are very different from hES cells" and "any cell line deriving from hEG cells will be dissimilar from cell lines derived from hES cells." See Declaration of September 21, 2004 (Declaration 1), para. 7-8.

Moreover, as stated by Dr. Benvenisty in the present Declaration (Declaration 2) "Thomson never satisfactorily verified that the human ES cell lines that were generated "maintained the potential to form derivatives of all three embryonic germ layers" (see Thomson p. 1146, 1st column, lines 30-32), because he relied on evidence of teratomas generated in SCID mice injected with said cell lines (*id.*, p. 1147, Figure 4) to conclude that his cells actually were pluripotent human ES cells. As pointed out by Shamblott on the first page of the cited reference (see Shamblott, p. 13726, col. 2) "ES and EG cells from some species can form teratocarcinomas when injected into histocompatible or immunologically comprised mice. This property alone may not be a definitive test of stem cell pluripotency, as it has been demonstrated that rat and mouse visceral (yolk sac) endoderm are capable of forming highly differentiated teratomas containing cells of all three embryonic germ layers." Nonetheless, assuming Thomson's cells actually are pluripotent hES cells, if one starts with Thomson's cells, and tries to apply the methods of Shamblott for hEG cells to form hEBs, and then use mouse protocols such as Yuen et al. to direct differentiation, one will not get the results we have seen, not would one in the art even combine these technologies." See Declaration 2, para. 3.

And as first pointed out by Dr. Benvenisty in Declaration 1 and reiterated in Declaration 2, para. 4, "the human EG cells in Shamblott are very different from hES cells, with respect to many features. Any cell line deriving from hEG cells will be dissimilar from cell lines derived from hES cells." In fact, human EG cells are so different from hES cells that Shamblott admits on p. 1372, col. 1, para. 1 of the Results that "Unlike mouse pluripotent stem cells (ES and EG), these human cells were more resistant to disaggregation by trypsin/EDTA-based reagents" and again in col. 2, para. 3 of the Discussion that "The highly compacted nature of these colonies suggests strong cell-cell adhesion. These interactions are *notably* more resistant to trypsin than mouse ES and EG colonies. *Alternative* disaggregation enzymes are currently under investigation" (emphasis added).

As amended, claims 8, 48, 55, 60 and new claims 70, 71 and 73 require the human EBs to be chemically dissociated and then the ES cells are cultured as a monolayer. This is not possible with Shamblott's EG cells. As emphasized by Dr. Benvensity, "More importantly, my methods for forming hEBs and directing

differentiation of human embryonic cells derived from those hEBs *require* dissociation of the hES cells initially used, followed by aggregation to hEBs, followed by additional dissociation of the hEBs to obtain dissociated human embryonic cells that can be treated with various factors to direct differentiation." See Declaration, para. 4.

In addition, as stated in the Response and Declaration 1 of September, 2004, Dr. Benvenisty is the first to successfully show how one can direct differentiation in human ES cells derived from human EBs (see also Declaration 2, para. 5). Moreover, the prior art was extremely unpredictable until the Applicant's remarkable breakthrough. At the time the presently claimed invention was filed, efforts by persons skilled in the art to show *in vitro* formation of human EBs had failed, and efforts to form EBs from monkey ES cells (Rhesus and marmoset) had proven impossible or sporadic at best, never mind taking it all a step further to direct differentiation of hES cells derived from human EBs. As pointed out by Dr. Benvenisty "Without the ability to start with actual pluripotent human ES cells, dissociate the hES cells and allow them to aggregate and form true human EBs, and then dissociate the hEBs into dissociated human embryonic cells, one would never be able to look to Yuen et al. and mouse protocols for directing differentiation of the dissociated EB-derived human embryonic cells." See Declaration 2, para. 6

Also, as stressed by Dr. Benvenisty "This second dissociation step is essential for directing differentiation of human embryonic cells derived from hEBs. For these reasons, it was not obvious to produce differentiated cells from human EBs and the establishment of embryoid bodies from human ES cells may be considered a new and not obvious technology since it was developed at a time when all the research reported that such technology was not possible (Reubinoff et al, 2000). As stated in Declaration 1, when I attended a conference in 2001 and presented my results showing human embryoid body formation from human ES cells and subsequent directed differentiation of the human embryonic cells, and many experts in the field simply did not believe me (including Reubinoff), questioning my results intensely. Therefore, the combination of Thomson, Shamblott and Yuen would never be made by someone on the field trying to form true human EBs and then dissociate them to dissociated human embryonic cells that can be

treated with various factors to direct differentiation. And even if such a combination were attempted, it would not be successful." See Declaration 2, para.8.

Therefore, one of ordinary skill in the art would not combine Thomson (hES cells) with Shamblott (hEG cells) and then Yuen (mouse EBs that are further dissociated, cultured and differentiated) because there is no expectation of success, one of the three elements required for a *prima facie* case of obviousness (see MPEP s. 2142). Basically, the combination of Thomson, Shamblott and Yuen would not lead to the presently claimed invention.

Elsewhere in the Office Action the Examiner rejects claims 8-12, 14-16, 48, 51 and 52 as being unpatentable over Thomson et al. in view of Shamblott et al. and further in view of Bain et al. (1995) *Devel. Biol.* **168**, 342-357 (see Office Action, p. 4); rejects claims 8, 11, 13, 48, 51 and 52 as being unpatentable over Thomson et al. in view of Shamblott et al. and further in view of Bain et al. and Wobus et al. (1988) *Biomed. Biochim. Acta* 47, 965-973 (see Office Action, p. 5); and rejects claim 55 as being unpatentable over Thomson et al. in view of Shamblott et al. and further in view of Bain et al. and Wobus et al. (see Office Action, p. 7).

The failings of Thomson et al. in combination with Shamblott et al. and Yuen et al. have been described above. Relative to the addition of Bain et al., and Wobus et al. without the primary combination of Thomson, Shamblott and Yuen, persons skilled in the art would not additionally combine Bain and/or Wobus. Moreover, Dr. Benvenisty stated previously in his Declaration of September 21, 2004 (Declaration 1), that "Bain reports the treatment of mouse ES cell-derived embryoid bodies with retinoic acid (RA) and their differentiation into the neuronal lineage. The technology of developing embryoid bodies from mouse ES cells and directing their differentiation was known and established at the time of the present invention. However, the techniques and protocols that applied for mouse ES cells were not adequate for human ES cells. Therefore, the establishment of embryoid bodies from human ES cells may be considered a new and not obvious technology, since it was developed at a time when all the research reported that such technology was not possible (Reubinoff et al, 2000)." See Declaration1, para. 18.

Similar considerations argue against additionally combining Wobus to the Thomson, Shamblott, Yuen combination (for a more detailed discussion of Wobus, see Declaration 1, para. 22, submitted September 21, 2004).

For all the foregoing reasons, Applicant respectfully submits that the pending claims are not obvious in light of the cited prior art. Reconsideration of the claims and withdrawal of the obviousness rejections are therefore requested.

CONCLUSION

Claims 1 – 7, 17 – 47, and 10 are cancelled, but Applicant reserves the right to pursue such claims in a later related application. Claims 49, 50, 53, 54, 56-59, 61-64 and 66-69 are withdrawn without prejudice, but withdrawn claims 53, 56, 58, 61, 63, 64, 66, and 68 are also amended, because Applicant requests consideration for allowance if the base claims upon which these withdrawn claims depend (i.e. claims 8, 48, 55 and 60) are found allowable.

In view of the arguments and amendments presented, Applicant respectfully submits that all pending claims are now in condition for allowance. Reconsideration of the claims and a notice of allowance are therefore respectfully requested.

Applicant believes that a two-month extension of time is required and petitions for such an extension of time. However, in the event that an additional extension is required, this conditional petition for an additional extension of time is requested. Therefore, please charge any fees required for the timely consideration of this application to deposit account number 19-4972.

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Respectfully submitted,

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